American Journal of Hematology 60:175–180 (1999)

Decreased Plasma Tissue Factor Pathway Inhibitor in Women Taking Combined Oral Contraceptives

Geoffrey M. Harris,[1] Caroline L. Stendt,[1] Beverley J. Vollenhoven,[2] T. Eng Gan,[1] and Peter G. Tipping[3]*

[1]Department of Haematology, Monash Medical Centre, Victoria, Australia
[2]Monash University Department of Obstetrics and Gynaecology, Monash Medical Centre, Victoria, Australia
[3]Monash University Department of Medicine, Monash Medical Centre, Victoria, Australia

Use of combined oral contraceptives (OC) is associated with a significant risk of thrombosis. The mechanisms of this effect are not clearly defined. Tissue factor pathway inhibitor (TFPI) is a circulating anti-coagulant that inhibits the earliest steps in activation of the extrinsic coagulation pathway. It plays a central role in control of coagulation but its contribution to the thrombotic risk associated with OC has not been assessed. Plasma TFPI antigen and activity, factor VIIa, prothrombin fragments 1&2, von Willebrand antigen, fibrinogen, and low density lipoprotein cholesterol were measured by standard assays in women taking OC (aged 16 to 45 years, n = 40) and age-matched women not taking OC (controls, n = 40). Plasma TFPI antigen did not vary significantly across the menstrual cycle in controls. Women on OC had a 25% reduction in plasma TFPI antigen (median 51.0 ng/ml; 95% confidence intervals [CI] 37.5 to 85.5; control 68.0 ng/ml, CI 61.0 to 95.0; $P < 0.001$) and a 29% reduction in TFPI activity (78.5 U/ml, CI 57.5 to 107.5; control 111.0 U/ml, CI 79.5 to 171.0; $P < 0.001$) compared to controls. Plasma factor VIIa activity and prothrombin fragments 1&2 were also significantly increased in women using OC (both $P < 0.001$), indicating activation of the extrinsic coagulation pathway. These results demonstrate that normal cyclic variations in estrogen and/or progesterone do not significantly alter plasma TFPI levels. However, estrogens and/or progestogens in OC result in activation of the extrinsic coagulation pathway and significantly reduce plasma TFPI, its major circulating inhibitor. Reduced plasma TFPI levels may underlie the thrombotic effects of OC. Am. J. Hematol. 60:175–180, 1999.

Key words: oral contraceptive; tissue factor pathway inhibitor; thrombosis; coagulation; factor VIIa; prothrombin; lipoprotein

INTRODUCTION

Increased risk of arterial and venous thrombosis is a well-recognized risk associated with use of the combined oral contraceptive (OC) pill, which has been confirmed with current OC formulations [1,2]. A minority of women who develop thromboembolism while taking OC have activated protein C resistance or factor V gene mutations [3]. Higher estrogen doses [4,5] and cigarette smoking [6] accentuate this risk. A number of changes in coagulation factors have been reported with OC use. The most consistently reported changes are an increase in factor VII activity and small increases in fibrinogen levels (reviewed in Bottinger et al. [4]). Decreased protein S levels [7] and enhanced fibrinolysis [8] have been reported; however, the mechanism by which these changes lead to the increased propensity for activation of coagulation is unclear.

Activation of coagulation in vivo is now considered to occur principally via the extrinsic coagulation pathway, which is initiated by binding of tissue factor (TF) with factor VII [9]. Initiation of coagulation is prevented under normal conditions by sequestration of TF from contact with plasma proteins and by the presence of a

*Correspondence to: Dr. Peter Tipping, Department of Medicine, 5th floor, Block E, Monash Medical Centre, 246 Clayton Rd., Clayton, 3168, Victoria, Australia. E-mail: peter.tipping@med.monash.edu.au

Received for publication 5 March 1998; Accepted 7 October 1998

Kunitz-type serine protease inhibitor, tissue factor pathway inhibitor (TFPI). TFPI is present in the plasma, platelets, and on the endothelial surface and inhibits the earliest steps in extrinsic pathway activation by binding factor Xa and TF/factor VIIa complexes in an inactive quaternary complex [10].

Little is known about the physiological regulation of TFPI. Plasma TFPI levels are reported to be positively correlated with low density lipoprotein (LDL) levels [11] and age [12]. Another report suggests that the age effect on plasma TFPI is restricted to the female population, who overall have lower levels than men [13]. Low plasma TFPI levels have been reported in patients with ischemic stroke [14] and with thrombotic thrombocytopenic purpura [15]. Increased levels have been reported in disseminated intravascular coagulation [16]. With the exception of heparin, the effects of therapeutic drugs on plasma TFPI levels have not been reported.

In the course of establishing a normal range for plasma TFPI, we noted low levels in several healthy women taking OC. This effect has not been reported previously and may have significant implications for understanding the increased thrombotic risk associated with OC use. This report describes the results of a study that aimed to determine whether use of OC is associated with significant changes in plasma TFPI which may contribute to their thrombotic risk.

MATERIALS AND METHODS

Blood Collection

Blood was collected by venipuncture directly into evacuated tubes, using a 21-gauge needle. The first 10-ml sample was drawn into a plain tube to obtain serum for lipoprotein assays, and while the needle remained in the vein, to minimize activation of coagulation, a second sample was drawn into 3.8% buffered trisodium citrate to obtain plasma for all other assays. Samples were drawn between 9 AM and midday and immediately centrifuged at 2,500*g* for 10 min; the serum and plasma were separated, aliquoted, and stored at −85°C.

TFPI Antigen (TFPI Ag)

Plasma TFPI was assayed by an ELISA that detects intact and truncated forms of TFPI as well as TFPI complexed with TF and factor VIIa (Imubind Total TFPI ELISA, American Diagnostica, Greenwich, CT). The lower limit of detection of this assay is 0.35 ng/ml and the inter- and intra-assay coefficients of variation are both 6.3%.

TFPI Activity

TFPI functional activity was assayed by its ability to neutralize TF/VIIa complexes using the method of Sandset et al. [17]. This method was modified by substitution of recombinant human TF (Innovin, Dade Diagnostics, Aguada, PR) for rabbit brain thromboplastin. Factor X, factor VII, factor Xa, and spectrozyme Xa were purchased from American Diagnostica. The inter- and intra-assay coefficients of variation are 8.6% and 9.2%, respectively.

TF Antigen (TF Ag)

TF Ag was assayed by ELISA (Imubind tissue factor ELISA, [American Diagnostica]). The lower limit of detection of this assay is 30 pg/ml and the inter-and intra-assay coefficients of variation are both <5.0%.

TF Activity

TF activity was measured by its ability to form an allosterically activate complex with factor VII, which cleaves a synthetic chromogenic substrate, using a commercial kit (Actichrome TF activity assay, American Diagnostica). Plasma samples were initially assayed at a dilution of 1 in 10 in 5% TF/TFPI depleted plasma as recommended. As activity was undetectable, the assay was repeated using undiluted samples. The lower limit of detection of this assay is 0.02 nM using samples diluted 1 in 10.

Prothrombin Fragments 1&2 (PF 1&2)

PF 1&2 were assayed by ELISA (Enzygnost F1+2, Behringwerke AG, Marburg, Germany) which detects a 14-amino acid sequence located within the carboxy-terminal portion of the F1+2 moiety [18]. The lower limit of detection of this assay is 0.04 nmol/L and the inter- and intra-assay coefficients of variation are 8.4% and 3.0%, respectively.

Factor VIIa

Activated factor VII was assayed by a clot based technique [19] utilizing a recombinant truncated TF that allows only factor VIIa and not factor VII to activate coagulation (Staclot VIIa-rTF, Diagnostica Stago, Asnieres-Sur-Seine, France). The lower limit of detection of this assay is 5 mU/ml and the inter- and intra-assay coefficients of variation are 6.8% and 8.1%, respectively.

von Willebrand Antigen (vW Ag)

vW Ag was assayed by an in-house ELISA using a rabbit anti-human vW Ag antibody (A082, Dakopatts, Copenhagen, Denmark) for capture and a peroxidase conjugated rabbit anti-vWAg antibody (P226, Dakoplatts) for detection. Results are expressed as percentage of antigen in a pool of plasma from 20 normal males. This assay can detect levels above 5% of normal and has inter- and intra-assay coefficients of variation of 8.6% and 5.0%, respectively.

Fibrinogen

Fibrinogen was quantified by the fibrin clot opacity method of Ellis and Stransky [20]. This assay has a sensitivity of 0.3 g/L and inter- and intra-assay coefficients of variation of 6.9% and 2.5%, respectively.

LDL Cholesterol

Plasma LDL cholesterol was calculated using the Friedrickson formula from the total cholesterol and the high density lipoprotein cholesterol, which were measured by standard techniques.

Study Design and Statistical Analysis

Experiments were performed in accordance with the Helsinki Declaration of 1975 as revised in 1983 and approved by the Human Experimentation Ethics Committee at Monash Medical Centre. Healthy women between the ages of 16 and 45 years were recruited from hospital and university staff and from a local general practice. Blood and relevant clinical details were collected after obtaining informed consent. In 18 women not taking the OC, sample collection was correlated with their menstrual cycle. These were 18 plasma samples collected in the basal phase (days 2 to 4), 17 in the mid-follicular phase (days 8 to 10), and 16 in the mid-luteal phase (days 20 to 22) of their cycles. The mean of the plasma TFPI measurements in each of these women was included in the control group data set.

Samples were analyzed for each parameter in a maximum of three batches. Results are expressed as the median and 5 to 95% confidence intervals (CI) where data is not normally distributed and as mean ± standard error of the mean (SEM) for normally distributed data. Statistical analysis was performed by the Mann-Whitney test, in which the population data failed a statistical test of normality and by the Student's *t*-test, in which the data was normally distributed. Multiple-group comparisons were performed by one way analysis of variance. These statistical calculations were performed using Sigma Stat, version 2 (Jandel Scientific, San Rafael, CA).

RESULTS

Group Demographics

Blood was collected from 43 women who were taking OC and 43 women not taking OC. Women who had commenced (n = 3) or ceased (n = 3) OC use within the past three months were excluded. The mean age of women in the two groups (OC 28.5 ± 1.2 years, n = 40; control 31.3 ± 1.3 years, n = 40) was not significantly different. All the women on OC were taking combined OC formulations containing ethinylestradiol (30 μg, n = 17; 35 μg, n = 7; or graded dosage of 30, 40, then 30 μg, n = 16) as the estrogen and either levonorgesterol (n = 32), norethisterone (n = 5), desogestrel (n = 1), or gestodene (n = 1) as the progestagen. None of the women in either group reported previous thrombotic or embolic disease, other significant diseases including diabetes, hypertension requiring treatment, or ischaemic heart disease, or regular use of pharmaceutical agents. Nine women in the OC group and eight women in the control group were smokers.

Effect of Normal Menstrual Cycle of Plasma TFPI Ag

TFPI Ag did not vary between phases of the menstrual cycle. TFPI Ag levels were 64.5 ng/ml (CI, 54.4 to 114.8) in the basal phase, 71.0 ng/ml (CI, 48.8 to 114.7) in the mid-follicular phase, and 67.0 ng/ml (CI, 42.6 to 84.4) in the mid-luteal phase. Although there was a trend toward higher TFPI levels in the mid-follicular phase of the cycle, these three groups were not statistically significantly different from each other or from the control group as a whole, by one way analysis of variance.

Effect of OC on TFPI Ag and Activity and Other Coagulation Parameters

TFPI Ag and activity were both significantly decreased in women taking the OC (Fig. 1). The extent of reduction in TFPI Ag (25%) and activity (29%) was similar. The decreased TFPI levels were associated with evidence of increased activation of the extrinsic coagulation pathway (Table I) with significant increases in plasma factor VIIa activity and in prothrombin fragments 1&2. Plasma levels of TF Ag were not different between the two groups and TF activity could not be detected in any specimen, when assayed either at a dilution of 1 in 10 or neat. There were no significant differences in vWAg or fibrinogen levels.

Effect of OC on Serum LDL Cholesterol

LDL cholesterol in women taking the OC (2.86 ± 0.16 mg/ml) was not significantly different from control (2.69 ± 0.14 mg/ml, $P = 0.42$). Thus, the decreased plasma TFPI levels observed in women using OC could not be attributed to effects on LDL cholesterol.

Effect of Age on TFPI Ag and Activity, Coagulation Parameters, and LDL Cholesterol (Table II)

Results were analyzed in two groups (those aged 15 to 30 years and those aged 31 to 45 years) to determine the effect of age on TFPI levels, other coagulation parameters, and LDL cholesterol. In women not taking OC (controls), there were no significant differences between the two age groups with respect to TFPI Ag or activity, factor VIIa, vW Ag, or fibrinogen levels. PF 1&2 showed a significant increase with age ($P = 0.013$) as did LDL cholesterol ($P = 0.007$). In women taking OC, TFPI Ag

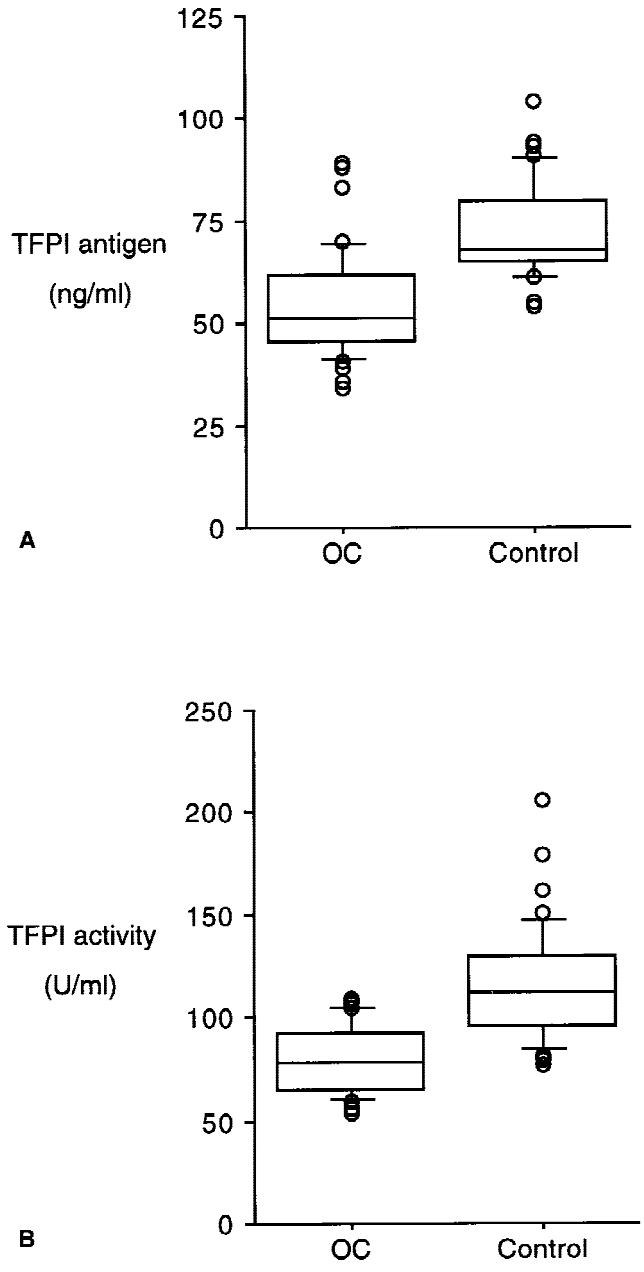

Fig. 1. Box plots demonstrating TFPI Ag (panel A) and TFPI activity (panel B) in women on OC and controls. Horizontal bars represent the 10th, 25th, 50th, 75th, and 90th percentile for each group, circles represent values below the 10th and above the 90th percentile. TFPI Ag and activity were both significantly lower in OC users than controls ($P < 0.001$).

and activity, TF Ag, factor VIIa, vW Ag, and fibrinogen levels were not influenced by age. PT 1&2 showed a significant increase with age ($P = 0.017$) but the increased LDL cholesterol did not reach statistical significance ($P = 0.067$). In each age group, differences in TFPI Ag, TFPI activity, factor VIIa, and PF between OC and controls remained significant.

TABLE I. Plasma TFPI Ag and Activity and Coagulation Parameters in Women Taking Combined OC and Women Not Taking OC (Control)*

	OC	Control	*P*
TFPI Ag (ng/ml)	51.0	68.0	<0.001
CI	37.5–85.5	61.0–95.0	
TFPI activity (U/ml)	78.5	111.0	<0.001
CI	57.5–107.5	79.5–171.0	
TF Ag (pg/ml)	90.0	97.0	$P = 0.43$
CI	29.0–183.5	42.9–233.6	
Factor Vlla (mU/ml)	54.0	28.0	<0.001
CI	23.5–258.0	16.9–52.4	
PF 1&2 (nmol/L)	1.05	0.8	<0.001
CI	0.50–2.10	0.43–1.21	
vW Ag (% normal)	93.5	80.0	$P = 0.089$
CI	52.5–191.0	50.0–157.0	
Fibrinogen (g/L)	2.20	2.20	$P = 0.099$
CI	1.85–3.16	1.80–2.86	

*TFPI, tissue factor pathway inhibitor; OC, oral contraceptives; Ag, antigen; PF, prothrombin fragments; vW, von Willebrand; CI, confidence interval.

DISCUSSION

This study shows significant effects of combined OC use on plasma TFPI Ag and function. Ethinylestradiol in doses between 30 and 50 μg per day in combination with a variety of progestagens resulted in a 25% decrease in TFPI Ag and 29% decrease in activity. Use of OC by younger women resulting in lower TFPI levels may explain the previously reported effect of age on plasma TFPI levels which is confined to women [13]. It is possible that declining levels of estrogen in older women may also contribute to this effect.

Evidence for the functional importance of this change in plasma TFPI was provided by the marked increase in factor VIIa activity. TF is an essential co-factor for generation of factor VIIa from factor VII by the enzymatic activity of minute quantities of factor Xa or IXa. Factor VIIa has a long circulating half-life similar to that of factor VII [21,22] and is slowly inactivated by antithrombin III [23]. The increased factor VIIa activity was observed in the absence of changes in plasma TF Ag. Thus, decreased TFPI, the major physiological inhibitor of factor VIIa activity, rather than increased TF may be an important determinant of the increase in factor VIIa activity in women on OC. Increased plasma factor VII concentration may also contribute to the 93% increase in factor VIIa activity in OC users.

Physiological coagulation in vivo is initiated by TF/factor VIIa complexes which activate factor X and factor IX. This leads to generation of a prothrombinase complex that cleaves prothrombin to thrombin. The presence of PF 1&2 in plasma is a sensitive index of activation of coagulation. In women taking OC, the significantly increased levels of PF provides evidence of activation of the extrinsic coagulation pathway. This, together with the

TABLE II. Effects of Age on Plasma TFPI Ag and Activity, Coagulation Parameters, and LDL Cholesterol in Women Taking Combined OC and Women Not Taking OC (Control)[†]

	OC		Control	
	16 to 30 years	31 to 45 years	16 to 30 years	31 to 45 years
Age (years)(mean ± SEM)	24.3 ± 0.7[a]	38.3 ± 0.9[a]	23.1 ± 0.9	37.9 ± 0.8
n	28	12	18	22
TFPI (ng/ml)	51.5[b,d]	48.5[c]	68.0[d]	68.5
CI	(38.5–70.9)	(36.5–88.4)	(57.4–96.8)	(61.0–94.8)
TFPI activity (U/ml)	81.5[b,d]	77.5[b]	104.5[d]	117.0
CI	(58.1–108.1)	(56.1–96.6)	(83.8–165.2)	(78.8–179.6)
TF Ag (pg/ml)	89[a,d]	94[a]	97[d]	95
CI	(28–208)	(33–170)	(32–156)	(52–262)
Factor Vlla (mU/ml)	47.5[b,d]	72.5[b]	27.5[d]	31.0
CI	(22.7–248.6)	(43.7–269.4)	(16.8–64.4)	(17.1–51.6)
PF 1&2 (nmol/L)	1.00[c,d]	1.38[b]	0.67[e]	0.91
CI	(0.454–1.79)	(0.78–2.20)	(0.34–1.19)	(0.62–1.26)
vW Ag (% normal)	93.58[a,d]	96.5[a]	76.5[d]	82.5
CI	(52.9–172.6)	(37.2–203.0)	(49.8–148.4)	(50.8–162.0)
Fibrinogen (g/L)	2.30[a,d]	2.20[a,b]	2.10[d]	2.20
CI	(1.99–3.22)	(1.80–2.99)	(1.84–2.83)	(1.80–2.84)
LDL cholesterol (mg/ml) (mean ± SEM)	2.68.18[a,d]	3.34 ± 0.32[a]	2.28 ± 0.18[e]	3.02 ± 0.18

[†]See Table I.
[a]Not significantly different from age-matched control.
[b]Significantly different from age-matched control ($P < 0.001$).
[c]Significantly different from agr-mathced control ($P = 0.009$).
[d]Not significantly different from OC or control-matched older age group.
[e]Significantly different from OC or control-matched older age group ($P = 0.013$ or less).

high levels of factor VIIa activity, indicates important functional consequences of these reduced TFPI levels. In the current study, no women taking OC had previous clinical evidence of thrombosis. However, increased risk of coagulation and clinically significant thrombosis associated with OC use is well established in other studies [1–7].

As similar changes in TFPI Ag and activity are seen in women taking OC, it is likely that the changes in TFPI function are directly related to reduced plasma protein concentrations. The cause of this reduced plasma TFPI Ag is unclear. TFPI is predominantly synthesized by endothelial cells [24] and is distributed between different pools in the circulation. A significant amount of TFPI is bound to glycosaminoglycans on the surface of endothelial cells and is released by heparin [25]. In the plasma, TFPI exists in a free form and bound to various lipoprotein fractions, mainly LDL. Thus, changes in synthesis and clearance, endothelial cell damage and lipoprotein effects may potentially alter plasma TFPI levels.

A significant amount of TFPI in plasma is associated with LDL and may be significantly increased in hypercholesterolemia [17]. OC have been reported to have variable effects on lipoprotein profiles depending on the hormonal combination and dose [26,27]. Formulations containing ethinylestradiol appear to be associated with increased LDL cholesterol. In the current study, the trend to increased LDL levels in women taking OC would increase rather than decrease plasma TFPI and could not account for the effects of OC on TFPI.

Variable effects on plasma TFPI levels have been reported in diseases associated with diffuse endothelial injury. In disseminated intravascular coagulation TFPI levels have been reported to be increased [16]; however, in thrombotic thrombocytopenia purpura, decreased TFPI levels have been reported [15]. OC are not known to cause endothelial injury. Supporting this, plasma levels of vW Ag, a sensitive index of endothelial injury, were not significantly different between OC users and controls in the current study. Release of bound TFPI due to endothelial cell injury would be expected to increase plasma TFPI levels and should not contribute to the decrease in TFPI in OC users.

Thus, it is likely that effects on TFPI synthesis or clearance are responsible for the decrease plasma TFPI levels. Very little is known about control of TFPI synthesis. TFPI synthesis by endothelial cells in vitro does not appear to be regulated by endotoxin or proinflammatory cytokines such as interleukin 1 and tumor necrosis factor-α [24], which regulate TF and plasminogen activator inhibitor-1 production. Mechanisms of TFPI clearance are also poorly defined. Clearance from plasma oc-

curs predominantly via hepatic uptake [28]. In vitro, uptake of TFPI by hepatoma cells occurs via the LDL receptor and a second receptor [24] and requires the carboxy terminus of the molecule [29]. The factors that control TFPI synthesis and clearance are poorly understood and effects of steroid hormones on these processes have not been reported.

In summary, these studies demonstrate a significant decrease in plasma TFPI levels associated with OC use. The functional significance of this effect is demonstrated by the increase in factor VIIa activity and PF, indicating activation of the extrinsic coagulation pathway. Changes in endothelial and lipoprotein pools of TFPI are unlikely to explain the effects of OC and effects on synthesis and/or clearance appear likely. Changes in plasma TFPI levels may be an important factor in the procoagulant and thrombotic risks associated with OC use.

ACKNOWLEDGMENTS

We thank Sr. N. Taylor for her assistance in recruiting subjects and collecting blood samples, Ms. Erica Malan for performing TF assays, and the Department of Clinical Biochemistry at Monash Medical Centre for determining LDL cholesterol levels. TFPI Ag ELISA kits were donated by American Diagnostica.

REFERENCES

1. World Health Organisation Collaborative Study of Cardiovascular Disease and Steroid Hormone Contraception. Venous thromboembolic disease and combined oral contraceptives: results of international multicentre case-control study. Lancet 1995;346:1575.
2. Farmer RDT, Lawrenson RA, Thompson CR, Kennedy JG, Hambleton IR. Population-based study of risk of venous thromboembolism associated with various oral contraceptives. Lancet 1997;349:83.
3. Bokarewa MI, Falk G, Sten-Linder M, Egberg N, Blombäck M, Bremme K. Thrombotic risk factors and oral contraception. J Lab Clin Med 1995;126:294.
4. Bottinger LE, Boman G, Eklunk G, Westerholm B. Oral contraceptives and thromboembolic disease: effects of lowering oestrogen content. Lancet 1980;i:1097.
5. Meade TW, Greenberg G, Thompson SG. Progersterones and cardiovascular reactions associated with oral contraceptives and a comparison of the safety of the 50 and 30 μg oestrogen preparations. Br Med J 1980;280:1157.
6. Royal College of General Practitioners' Oral Contraceptive Study. Further analysis of mortality in oral contraceptive users. Lancet 1981;i: 541.
7. Speroff L, De Cherney A, and the Advisory Board for New Progestins. Evaluation of a new generation of oral contraceptives. Obstet Gynaecol 1993;81:1034.
8. Malm J, Laurell M, Dahlback B. Changes in the plasma levels of vitamin K-dependent proteins C and S and of C4b-binding protein during pregnancy and oral contraceptive. Br J Haematol 1988;68:437.
9. Nemerson Y. The tissue factor pathway of blood coagulation. Semin Hematol 1992;29:170.
10. Broze GJ Jr. The role of tissue factor pathway inhibitor in a revised coagulation cascade. Semin Haematol 1992;29:159.
11. Zitoun D, Bara L, Basdevant A, Samama MM. Levels of factor VIIC associated with decreases tissue factor pathway inhibitor and increased plasminogen activator inhibitor-1 in dyslipidemias. Arterioscler Thromb Vasc Biol 1996;16:77.
12. Jespersen J, Petersen KR, Skouby SO. Effects of newer oral contraceptives on the inhibition of coagulation and fibrinolysis in relation to dosage and type of steroid. Am J Obstet Gynecol 1990;163:396.
13. Ariens RA, Coppola R, Potenza I, Mannucci PM. The increase with age of the components of the tissue factor coagulation pathway is gender dependent. Blood Coagul Fibrinolysis 1995;6:433.
14. Abumiya T, Yamaguchi T, Terasaki T, Kokawa T, Kario K, Kato H. Decreased plasma tissue factor pathway inhibitor activity in stroke patients. Thromb Haemost 1995;74:1050.
15. Kobayashi M, Wada H, Wakita Y, Shimura M, Nakase T, Hiyoyama K, Nagaya S, Minami N, Nakano T, Shiku H. Decreased plasma tissue factor pathway inhibitor levels in patients with thrombotic thrombocytopenic purpura. Thromb Haemost 1995;73:10.
16. Shimura M, Wada H, Wakita Y, Nakase T, Hiyoyama K, Nagaya S, Mori Y, Shiku H. Plasma tissue factor and tissue factor pathway inhibitor levels in patients with disseminated intravascular coagulation. Am J Hematol 1996;52:165.
17. Sandset PM, Larsen ML, Abildgaard U, Lindahl AK, Odegaard OR. Chromogenic substrate assay of extrinsic pathway inhibitor (EPI): levels in the normal human population and relation to cholesterol. Blood Coagul Fibrinolysis 1992;2:713.
18. Pelzer H, Schwarz A, Stuber W. Determination of human prothrombin activation fragment 1&2 in plasma with an antibody against a synthetic peptide. Thromb Haemost 1991;65:153.
19. Morrisey JH, Macik BG, Neuenschwander PT, Comp PC. Quantitation of activated factor VII levels in plasma using a tissue factor mutant selectively deficient in promoting factor VII activity. Blood 1993;81: 734.
20. Ellis BC, Stransky A. A quick and accurate method for the determination of fibrinogen in plasma. J Lab Clin Med 1961;58:477.
21. Seligsohn U, Kasper CK, Osterud, Rapaport SI. Activated factor VII: presence in factor IX concentrates and persistence in the circulation after infusion. Blood 1979;53:828.
22. Lindley CM, Sawyer WT, Macik BG, Lusher J, Harrison JF, Baird-Cox K, Birch K, Glazer S, Roberts HR. Pharmacokinetics and pharmacodynamics of recombinant factor VIIa. Clin Pharmacol Ther 1994; 55:638.
23. Kondo S, Kisiel W. Regulation of factor VIIa activity in plasma: evidence that antithrombin III is the sole plasma protease inhibitor of human factor VIIa. Thromb Res 1987;46:325.
24. Ameri A, Kuppuswamy MN, Basu S, Bajaj SP. Expression of tissue factor pathway inhibitor by cultured endothelial cells in response to inflammatory mediators. Blood 1992;79:3219.
25. Sandset PM, Abildgaard U, Larsen ML. Heparin induces release of extrinsic pathway inhibitor (EPI). Thromb Res 1988;50:803.
26. Krauss RM, Burkman RT. The metabolic impact of oral contraceptives. Am J Obstet Gynecol 1992;167:1177.
27. Wahl P, Walden C, Knopp R, Hoover J. Effect of oestrogen/progestin potency on lipid/lipoprotein cholesterol. N Engl J Med 1983;308:862.
28. Narita M, Bu G, Olins GM, Higichi DA, Herz J, Broze GJ Jr, Schwartz AL. Two receptor systems are involved in the plasma clearance of tissue factor pathway inhibitor in vivo. J Biol Chem 1995;270:24800.
29. Warshawsky I, Bu G, Mast A, Saffitz JE, Broze GJ Jr, Schwartz AL. The carboxy terminus of tissue factor pathway inhibitor is required for interacting with hepatoma cells in vitro and in vivo. J Clin Invest 1995;95:177.